

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :  
Masataka NADAOKA et al. : Attn: BOX PCT  
Serial No. NEW : Docket No. 2001-1915A  
Filed January 7, 2002 :

CHROMATOGRAPHY MEASURING  
METHOD  
[Corresponding to PCT/JP01/03840  
Filed May 8, 2001]

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents,  
Washington, DC 20231

Sir:

Prior to examination of the above-referenced U.S. patent application please amend the application as follows:

IN THE SPECIFICATION

Please amend the specification as follows:

Please replace the paragraph beginning at page 2, line 16, to page 3, line 8, with the following rewritten paragraph:

When a required amount of inspection target solution is applied to this immuno-chromatographic sensor, the inspection target solution elutes the marked antibody while permeating porous materials. Then, an amount of the marked antibody, which is the amount of the marked antibody eluted by the inspection target solution being bonded to the antibody in the reagent immobilization part, in the reagent immobilization part is measured, thereby detecting components to be measured in the inspection target solution. Further, the amount of the bonded marked antibody can be measured by visually confirming the amount of the marker such as gold

colloid particles, which remains behind as a result of the bonding of the marked antibody to the reagent immobilization part. That is, since the degree of coloration (the depth of a color) in the reagent immobilization part varies with the concentration of the antigen (components to be measured) included in the inspection target solution, the inspector visually checks this, thereby enabling the measurement.

**Please replace the paragraph beginning at page 15, line 15, to page 16, line 3, with the following rewritten paragraph:**

Further, the amount of the eluted marker reagent is measured before the measurement of the bonding amount of the marker reagent in the antibody immobilization part 4 (on the upstream). This is because after the inspection target solution is applied in the measurement operation, the inspection target solution develops on the antibody immobilization film 3 while it passes through the marker reagent hold part 2 before passing through the antibody immobilization part 4 and the marker reagent components is eluted, whereby when the amount of the eluted marker reagent is previously measured before the marker reagent passes through the antibody immobilization part 4 as a measurement part, it can promptly reflect the measurement of the amount of the marker reagent bonded in the antibody immobilization part 4.

#### **IN THE CLAIMS**

**Please amend the claims as follows:**

3. (Amended) The chromatography measuring method as defined in Claim 1, wherein  
the measurement of the amount of the eluted marker reagent components or residual marker reagent components which have not been eluted employs an optical detector.
4. (Amended) The chromatography measuring method as defined in Claim 1, wherein

the measurement of the amount of the eluted marker reagent components is performed in a part other than the reagent immobilization part.

5. (Amended) The chromatography measuring method as defined in Claim 1, wherein

the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

**Please add the following new claims:**

6. The chromatography measuring method as defined in Claim 2, wherein the measurement of the amount of the eluted marker reagent components or residual marker reagent components which have not been eluted employs an optical detector.

7. The chromatography measuring method as defined in Claim 6, wherein the measurement of the amount of the eluted marker reagent components is performed in a part other than the reagent immobilization part.

8. The chromatography measuring method as defined in Claim 7, wherein the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

9. The chromatography measuring method as defined in Claim 6, wherein the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

10. The chromatography measuring method as defined in Claim 2, wherein the measurement of the amount of the eluted marker reagent components is performed in a part other than the reagent immobilization part.

11. The chromatography measuring method as defined in Claim 10, wherein the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

12. The chromatography measuring method as defined in Claim 2, wherein the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

13. The chromatography measuring method as defined in Claim 3, wherein the measurement of the amount of the eluted marker reagent components is performed in a part other than the reagent immobilization part.

14. The chromatography measuring method as defined in Claim 13, wherein the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

15. The chromatography measuring method as defined in Claim 3, wherein the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

16. The chromatography measuring method as defined in Claim 4, wherein the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.

### REMARKS

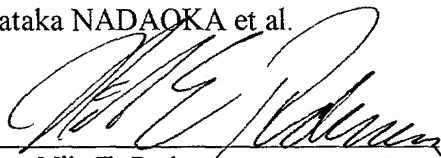
The present Preliminary Amendment is submitted to make minor editorial changes so as to generally improve the form of the specification and to delete the multiple dependency of the claims, thereby placing such claims in condition for examination and reducing the required PTO filing fee.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current Preliminary Amendment. The attached page is captioned "Version With Markings to Show Changes Made".

Respectfully submitted,

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state is included in another part thereof. When the inspection target solution is developed, bonding occurs in the reagent immobilization part and then the bonding amount is measured, whereby components to be measured in the inspection target solution can be measured.

This immuno-chromatographic sensor is generally constituted by an inspection target solution application part where an inspection target solution is applied and plural development layers, in which an antibody is immobilized in a reagent immobilization part, which is a part of the development layer, and an antibody marked with a marker such as gold colloid particles is held in a marker reagent hold part which is upstream of the reagent immobilization part in the development layer, in a dry state where it can be eluted by the inspection target solution.

When a required amount of inspection target solution is applied to this immuno-chromatographic sensor, the inspection target solution elutes the marked antibody while permeating porous materials. Then, an amount of the marked antibody, which is the amount of the marked antibody eluted by the inspection target solution being bonded to the antibody in the [marker]<sup>reagent</sup> immobilization part, in the reagent immobilization part is measured, thereby detecting components to be measured in the inspection target solution. Further, the amount of the bonded marked antibody can be measured by visually confirming the

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amount of the marker such as gold colloid particles, which remains behind as a result of the bonding of the marked antibody to the reagent immobilization part. That is, since the degree of coloration (the depth of a color) in the reagent immobilization part varies with the concentration of the antigen (components to be measured) included in the inspection target solution, the inspector visually checks this, thereby enabling the measurement.

While the description has been given here of a case where a sandwich reaction of an antigen antibody reaction is taken as a measurement principle, the measurement result can be also obtained in a bonding state of the marker reagent in an antibody immobilization part even when other competitive reactions are similarly taken as measurement principles.

Further, while in the above-described example the description is given of a case where the measurement result is visually obtained by a qualitative judgement, Japanese Published Patent Application No. Hei.8-334511 describes a method by which the degree of coloration in the reagent immobilization part of a specimen is imaged by a CCD to be judged automatically so as to improve a low reproducibility and individual variations of the visual judgement. In a case where a semi-quantitative or more accurate judgement is required as the measurement result, there are a method for reading by a transparent mode employing an optical reading device, which is

antibody immobilization part 4.

Figure 2(c) illustrates a state of the immuno-chromatographic sensor at the same point of time as shown in figure 2(b). In the figure, numeral 8 denotes an area where the residual amount of the marker reagent is measured.

As described above, the measurement of the amount of eluted marker reagent components is performed in an area other than the antibody immobilization part 4. This is because the bonding amount of the marker reagent varies with the amount of the measurement target in the inspection target solution in the antibody immobilization part 4, whereby when the marker reagent is to be read a specified time after the measurement is started, it cannot be judged whether or not its value results from the bonding reaction of the measurement target.

Further, the amount of the eluted marker reagent is measured before the measurement of the bonding amount of the marker reagent in the antibody immobilization part 4 (on the upstream). This is because after the inspection target solution is applied in the measurement operation, the inspection target solution develops on the antibody<sup>immobilization</sup> [reactive] film 3 while it passes through the marker reagent hold part 2 before passing through the antibody immobilization part 4 and the marker reagent components is eluted, whereby when the amount of the eluted marker reagent is previously measured before the marker reagent passes through the antibody

immobilization part 4 as a measurement part, it can promptly reflect the measurement of the amount of the marker reagent bonded in the antibody immobilization part 4.

Further, when there is no inconvenience even if such prompt correction is not performed, it is also possible that the amount of the marker reagent is measured in the marker reagent bonding amount measurement area 7 on the antibody immobilization part 4, and thereafter the measurement value makes reflect a measurement value obtained in the remaining marker reagent amount measurement area 8 in the state shown in Figure 2(c).

Hereinafter, the chromatography measuring method according to the first embodiment will be described in more detailed with reference to figures 1 to 6, taking quantity determination of hCG (human chorionin gonadotropin) in the urine as a specific example of the chromatography measuring method employing a biosensor according to the first embodiment. Here, the measurement method of the first embodiment is not restricted to the quantity determination of hCG.

First, as a specimen (immuno-chromatographic sensor) according to the first embodiment, there is manufactured an immuno-chromatographic specimen which includes an anti-hCG- $\beta$  antibody immobilization line and a broad band of a complex of an anti-hCG- $\alpha$  antibody and a gold colloid in a nitrocellulose film.

3. The chromatography measuring method as defined in Claim 1  
 [or 2] wherein

the measurement of the amount of the eluted marker reagent components or residual marker reagent components which have not been eluted employs an optical detector.

4. The chromatography measuring method as defined in <sup>claim 1</sup> [any of  
 Claims 1 to 3], wherein

the measurement of the amount of the eluted marker reagent components is performed in a part other than the reagent immobilization part.

5. The chromatography measuring method as defined in <sup>claim 1</sup> [any of  
 Claims 1 to 4], wherein

the measurement of the amount of the eluted marker reagent components is performed before the bonding amount of the marker reagent is measured in the reagent immobilization part.